PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Nuijten et al.

Serial No.: 09/749,025

Filed: December 27, 2000

For: SALMONELLA VACCINE

Confirmation No.: 6121

Examiner: V. Ford

Group Art Unit: 1645

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DECLARATION UNDER 37 C.F.R. § 1.132 OF DR. PETRUS NUIJTEN

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dr. Petrus Nuijten hereby declares:

1. I am a named inventor on the above-referenced patent application.
2. A copy of my curriculum vitae is attached.

3. I understand that in the Office Action mailed October 20, 2006, the Examiner has questioned the fact that the application is enabling for a vaccine composition for the protection against Salmonellosis comprising an immunologically effective amount of any *Salmonella* mutated bacterium wherein the mutated bacterium lack flagellin and wherein the mutated bacterium is attenuated. I also understand that the claims at-issue have been amended and no longer claim a "vaccine."

4. The as-filed specification is enabling because it includes working examples of non-flagellated mutant *Salmonella* compositions which reduce colonization rates in both chickens and pigs. Specifically, Example 3 of the specification demonstrates that chickens vaccinated with a non-motile mutant of *S. typhimurium* STMP, called *S. typhimurium* STM2000, had reduced colonization of the intestinal tract.

5. Example 4, at pages 22-23 of the specification, shows that the live attenuated flagella-less *S. typhimurium* STM2000 vaccine significantly reduced fecal shedding in pigs after a challenge infection with a wild-type *S. typhimurium* serotype.

6. The specification also provides detailed instructions for selecting non-motile mutants from serotype *S. typhimurium* SL3261. (Example 1, page 17). In this example, a flagellin protein gene of *S. typhimurium* SL3261 was chemically mutagenized with NTG and non-motile mutants were selected by light microscopy. The selected mutant was named STM2001 and subsequent electrophoretic analysis revealed that the mutant lacked the flagellin protein fragment of 51kDa and pI 4.7, as compared to the non-mutant parent serotype. *Id.*

7. Attached hereto, I present *in vivo* data using four different strains of *Salmonella enterica* bacteria (*S. typhimurium, S. enteritidis, S. anatum and S. hadar*) confirming reduced colonization of wild-type Salmonella in the cloacae of chickens after they were given *Salmonella enterica* fla^- strains. Therefore, it is submitted that the application provides the skilled person with sufficient guidance to make and use the claimed compositions.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United

States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: April ___, 2007

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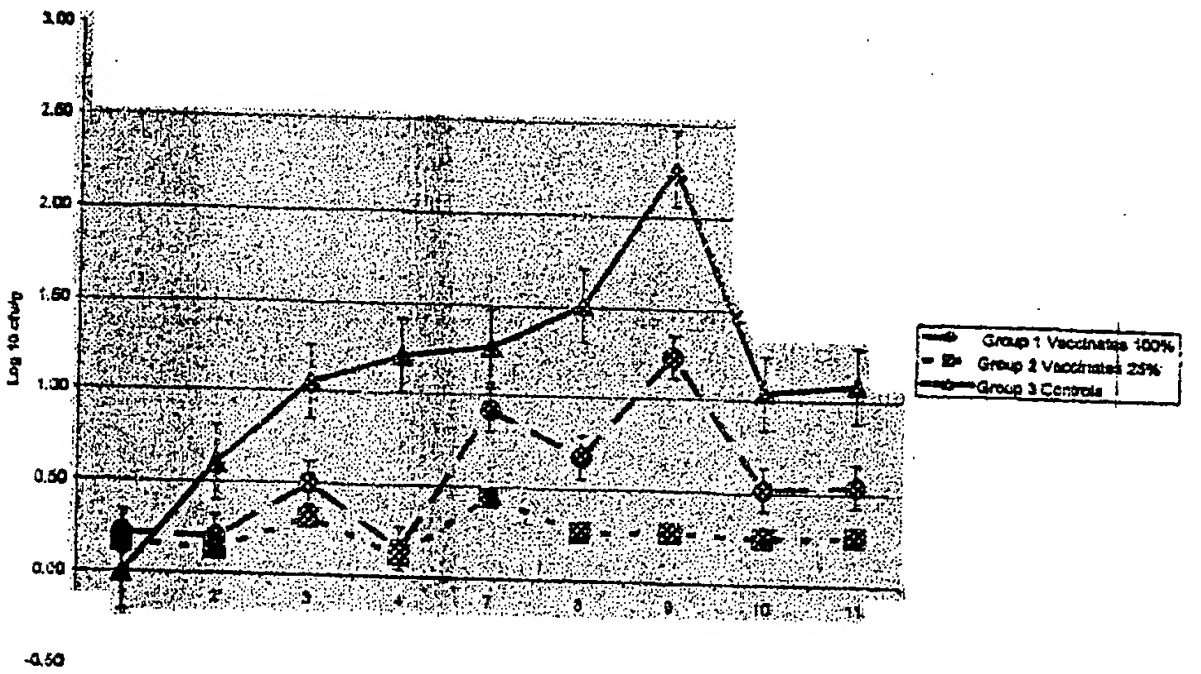
Dr. Petrus Nuijten

SUMMARY

Group 1 of 30 SPF layers were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising $2x10^9$ killed cells of each of FliC mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 3 (30 birds) was not vaccinated and Group 2 (30 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with 10^7 cfu of a different strain of wild type S. Anatum and this inoculation was repeated on the 2 subsequent days (i.e. 3 lots of 10^7 cfu over 3 days). Cloacal samples were tested for the presence of S. Anatum for 11 days. The extent of S. Anatum colonisation was expressed as the group mean number of S. Anatum cfu/g of cloacal sample. S. Anatum colonisation in group 1 (figure) was significantly less ($P<0.05$) than seen in group 3 (figure) or group 2. S. Anatum colonisation in group 2 was also significantly less than in group 3 (figure).

Figure 1. Numbers of S. Anatum recovered from cloacal swabs.



SUMMARY

Group 1 (28 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising $2x10^9$ killed cells of each of fliC mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 2 (28 birds) was not vaccinated and Group 3 (28 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with 10^9 cfu of a different strain f wild type S.Enteritidis. Cloacal samples were tested for the presence of S.Enteritidis for 14 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S.Enteritidis colonisation was expressed as the percentage of birds with different levels of contamination. S.Enteritidis colonisation in group 1 (figure 1) was significantly less ($P<0.05$) than seen in group 2 (figure 2) or group 3 (not shown: group 3 not significantly different form group 2).

Figure 1

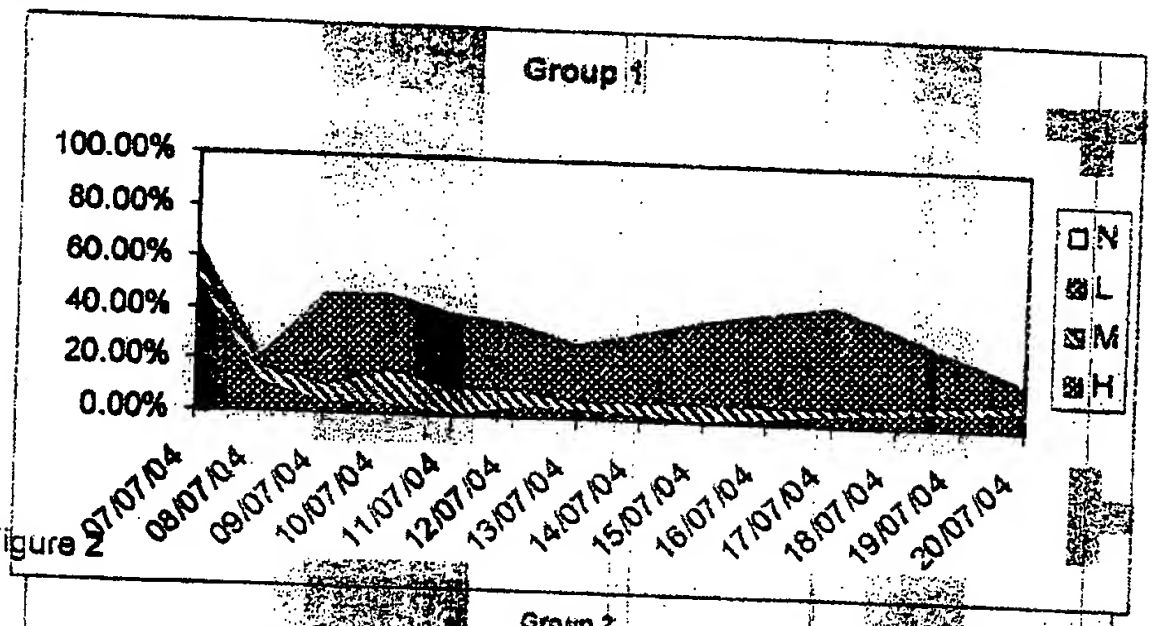
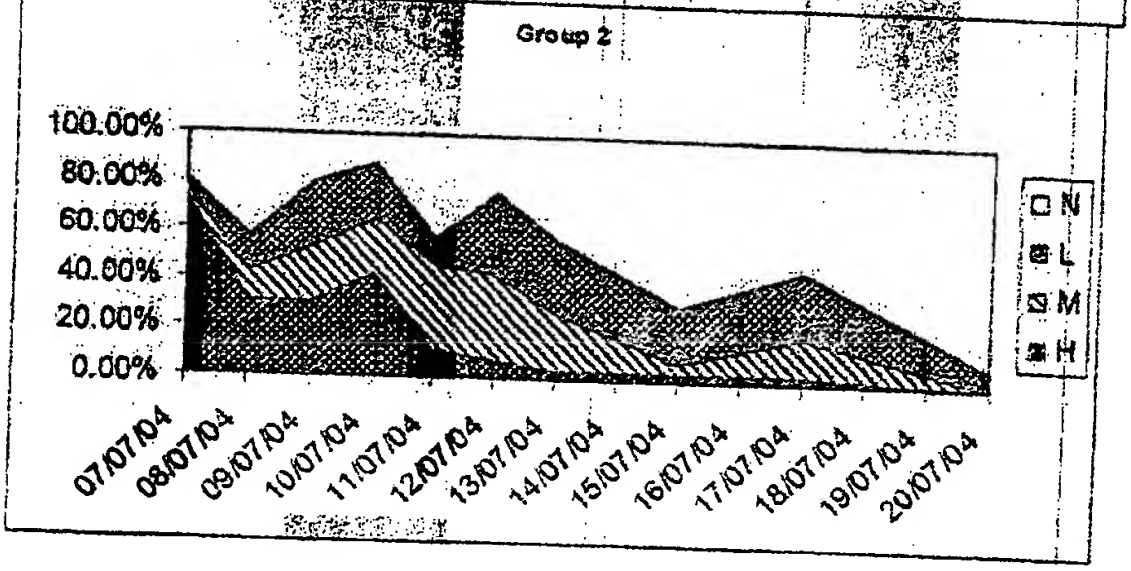


Figure 2



SUMMARY

Group 6 (28 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising $2x10^9$ killed cells of each of fliC mutants of S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 7 (28 birds) was not vaccinated and Group 8 (28 birds) was given a vaccine which contained 25% of the amounts of each antigen. At 12 weeks of age all birds were challenged orally with 10^9 cfu of a different strain of wild type S. Hadar. Cloacal samples were tested for the presence of S. Hadar for 14 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S. Hadar colonisation was expressed as the percentage of birds with different levels of contamination. S. Hadar colonisation in group 6 (figure 1) was significantly less ($P<0.05$) than seen in group 7 (figure 2) or group 8 (not shown: group 8 not significantly different form group 7).

Figure

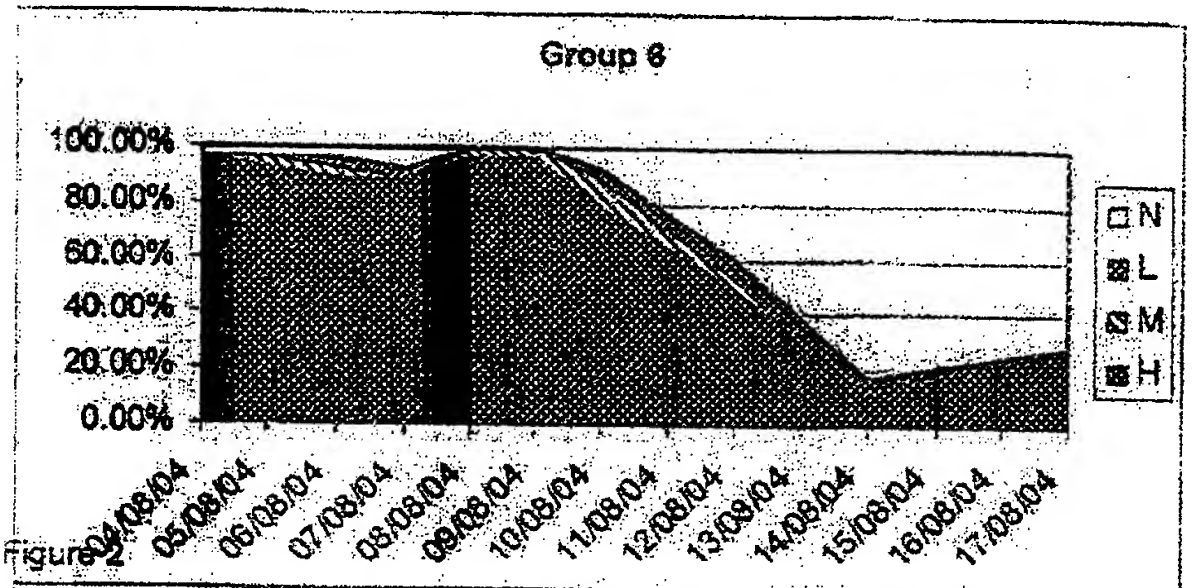
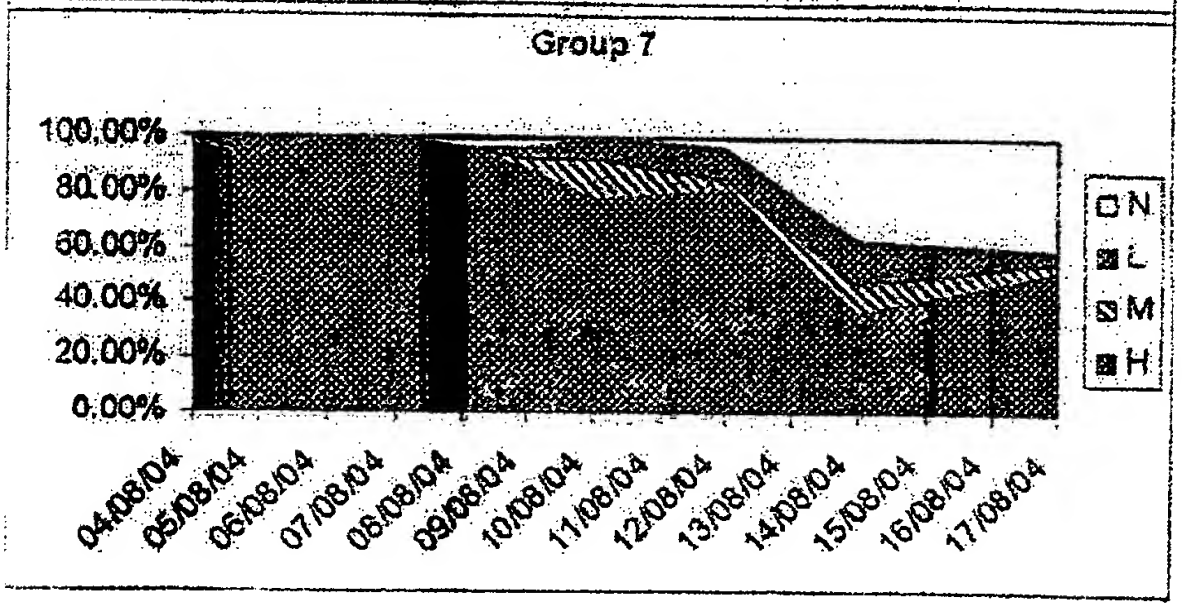


Figure 2



SUMMARY

Group 11 (14 SPF layers) were vaccinated i/m at 4 weeks of age and again at 8 weeks of age with an experimental vaccine comprising $2x10^9$ killed cells of each of fliC mutants S.Typhimurium, S.Enteritidis, S. Anatum and S. Hadar in 25% alhydrogel with thiomersal as preservative. Group 12 (14 birds) was not vaccinated. At 12 weeks of age all birds were challenged orally with 10^9 cfu of a different strain of wild type S.Typhimurium and this inoculation was repeated on the 2 subsequent days (i.e. 3 lots of 10^9 cfu over 3 days). Cloacal samples were tested for the presence of S.Typhimurium for 11 days. The amount of contamination recovered on culture medium was characterised as negative (N), low (L), medium (M) or high (H). The extent of S.Typhimurium colonisation was expressed as the percentage of birds with different levels of contamination. S.Typhimurium colonisation in group 11 (figure 1) was significantly less ($P<0.05$) than seen in group 12 (figure 2).

Figure 1

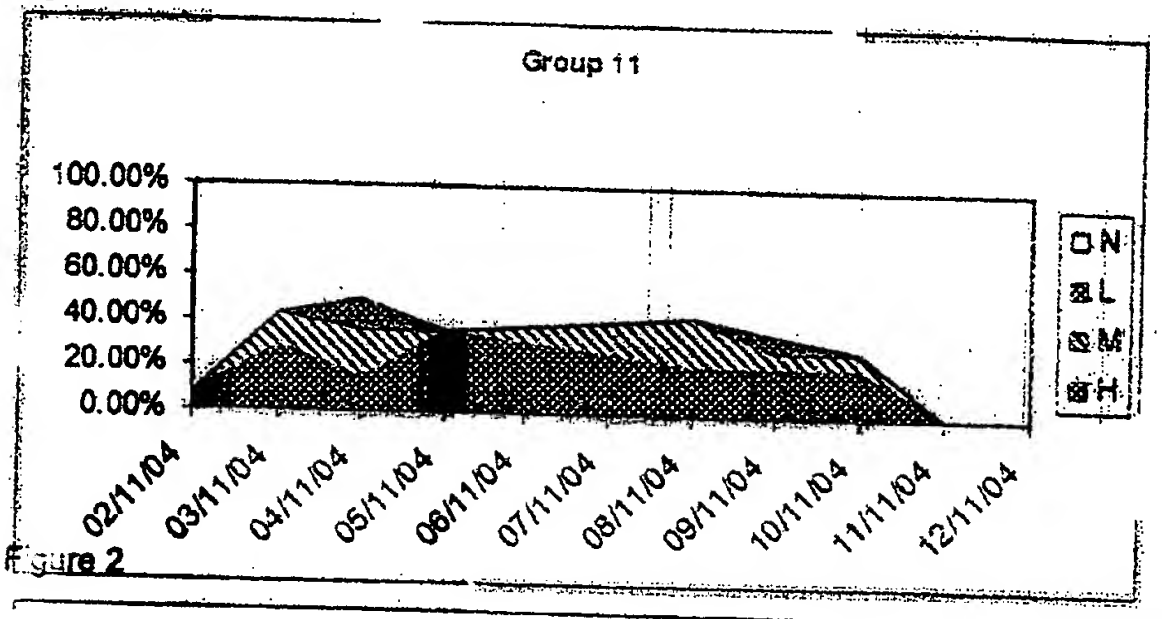


Figure 2